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Diagnostic Accuracy of Intra-Operative Squash Cytology for Spinal Lesions

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ABSTRACT
Squash cytology serves as a rapid intraoperative tool for the diagnosis of spinal lesions. However, the presence of fibrous elements and bony spicules can sometimes pose challenges to interpretation. Although another intraoperative diagnostic tool, frozen section, is available, the soft, friable nature and high-water content of tissue can hinder the preparation of good-quality frozen sections. This highlights the need to further explore the utility of squash cytology for the intraoperative diagnosis of spinal lesions. Our study emphasizes the importance of squash cytology in the diagnosis of spinal lesions. A retrospective analytical study was conducted at the Department of Neuropathology at a tertiary hospital in India. A total of 64 samples were collected for intraoperative squash cytology, followed by biopsies for histopathological correlation. Statistical analysis was performed, and various parameters like sensitivity, specificity, and diagnostic accuracy of squash cytology were calculated, with histopathology serving as the gold standard. Out of the 64 patients suspected of having spinal lesions, 39 cases (60.93%) were neoplastic. The mean age of presentation was 37.5 years, with no sex predilection. The most common spinal tumor was schwannoma (10 cases, 15.62%), followed by meningioma (6 cases, 9.37%). Concordance between squash cytology and histopathology was found in 60 cases (93.75%), with histopathology serving as the reference standard. The sensitivity, specificity, and diagnostic accuracy were 94.73%, 96.15%, and 95.31%, respectively. Squash cytology is a rapid, inexpensive, and reasonably accurate intraoperative diagnostic test for spinal lesions. When combined with clinical and radiological correlation, it demonstrates high diagnostic accuracy.

KEYWORDS: intra-operative diagnosis; spinal cord tumors; squash cytology

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1. Introduction

Squash cytology plays a crucial role in the intraoperative diagnosis of intracranial lesions, and its efficacy is well established. Its use was pioneered by Eisenhardt and Cushing in 1930, followed by Badt in 1937 [1, 2]. However, due to limited literature available on squash cytology of spinal lesions, its potential in diagnosing these lesions remains largely unexplored. Spinal lesions can be categorized as neoplastic or non-neoplastic and may originate from the spinal cord, filum terminale, sympathetic chain, meninges, intraspinal vessels, or vertebrae [3]. Squash cytology is valuable for differentiating between neoplastic and non-neoplastic processes initially and identifying the most frequently encountered neoplasms in this region. However, interpretation can be hindered by crushing, overstretching, cellular overlapping, the presence of fibrous elements, and bony spicules. Delays in diagnosing spinal lesions can lead to significant patient morbidity. Frozen section is another crucial intraoperative diagnostic tool, but freezing artifacts, soft and friable tissue nature, and high-water content often result in poor-quality sections. Squash cytology is gaining traction in cases involving spinal lesions, providing surgeons with valuable guidance for optimal postoperative treatment strategies until histopathological examination is complete. Our study aims to highlight the role of squash cytology in the intraoperative diagnosis of spinal lesions, analyze their cytomorphology, and explore their correlation with histopathology.

2. Materials and Methods

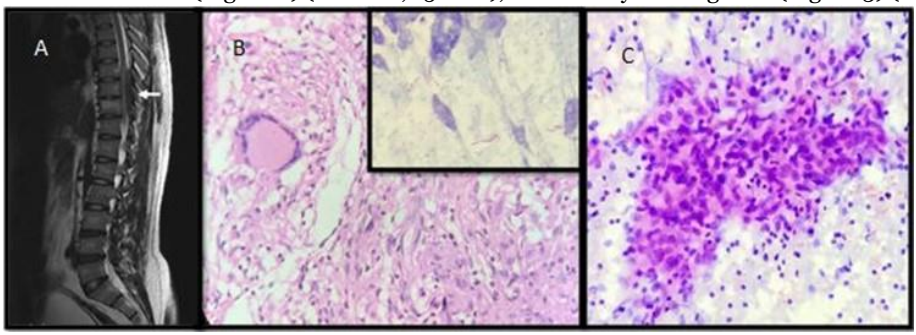
A retrospective analytical study was conducted at the Department of Neuropathology, in collaboration with the Department of Neurosurgery, at a tertiary care hospital in India. Data were collected from April 2022 to August 2023 after obtaining approval from the institute's ethical committee (ethical approval number: IEC/2023/10098). Informed consent was obtained from each patient for the collection and processing of samples.

All patients presenting to the neurosurgery department with a clinical and radiological diagnosis of a spinal space-occupying lesion and for whom both squash cytology and histopathology specimens were available were included in the study. Patients were selected through a random selection process. Cases with inadequate squash cytology or inadequate histopathology specimens were excluded from the study.

3. Results

Out of 64 patients clinically and radiologically suspected of having spinal space-occupying lesions, 39 cases (60.93%) were neoplastic and 25 cases (39.06%) were non-neoplastic. There were 34 male patients (53.12%) and 33 female patients (51.56%), with a male-to-female ratio of 1.03:1, indicating no sex predilection. The age range of the patients was 5 to 88 years, with a mean age of presentation at 37.5 years. The youngest patient was a 5-year-old female diagnosed with a malignant peripheral nerve sheath tumor, and the eldest was an 88-year-old male diagnosed with tuberculosis, confirmed by Ziehl-Neelsen (ZN) staining. The most common spinal tumor observed in our study

was schwannoma (Figure 2) (10 cases, 15.62%), followed by meningioma (Figure 3) (6



cases, 9.37%) and plasmacytoma (Figure 4) (3 cases, 4.68%). Schwannoma was the most common intradural extramedullary (IDEM) tumor, and ependymoma was the most common intramedullary tumor identified in our study. Schwannoma cases were

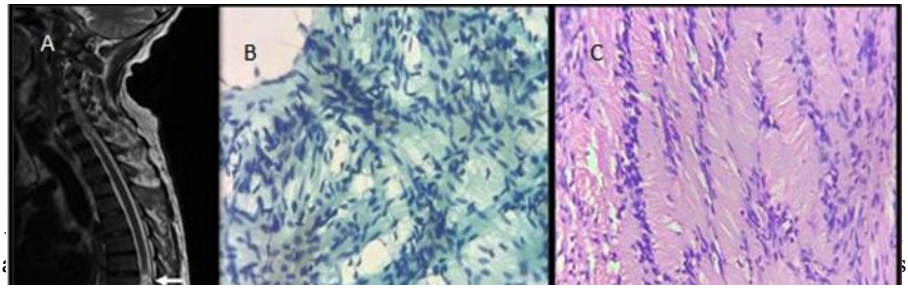


Figure 2. T2W sagittal MR image shows T2 hyperintense lesion in intradural extramedullary location at from D8-D9 level (arrow) (A); squash cytology smear shows cohesive sheets of benign spindle cells (B, PAP stain, x40); histology section shows hypercellular areas with palisading of cells (verocay bodies) alternating with hypocellular areas (C, HE stain, x40).

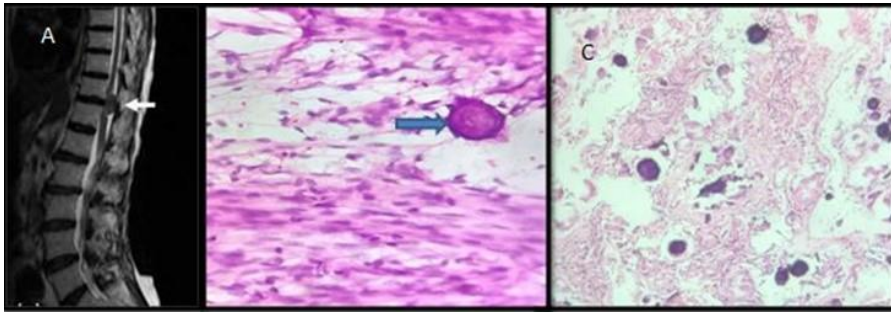


Figure 3. T2W sagittal MR image shows T2 hypointense lesion in intradural extramedullary location at from D11-12 level (arrow) (A); squash cytology smear shows syncytial sheets of cells with intranuclear pseudoinclusions and psammoma body (arrow) (B, PAP stain, x40); histology section shows neoplastic cells arranged in whorls along with plenty of psammoma bodies (C, HE stain, x40).

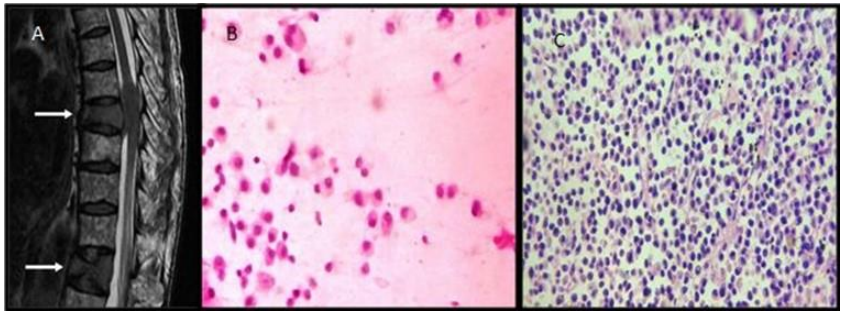


Figure 4. T2W sagittal MR image shows T2 hypointense lesion in D8 and D12 vertebra (arrows) with decreased height of D12 vertebra and posterior bulging of soft tissue at D8 level causing indentation of thecal sac (A); squash cytology smear shows monotonous population of neoplastic cells with round eccentric nuclei and perinuclear hof (arrow) (B, HE stain, x40); histology section shows sheets of neoplastic plasma cells (arrow) infiltrating the bone (C, HE stain, x40).

more prevalent in the 4th decade, while meningioma cases were more common in the 5th decade. Table 1 presents a breakdown of the spinal lesions based on squash cytology results. Concordance between squash cytology and histopathology was found in 60 cases (93.75%) out of 64 patients studied, as shown in Table 2. Four cases (6.25%) were found discordant. The true positive, true negative, false positive, and false negative cases are detailed in Table 3. Based on histopathology as the gold standard, the sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of squash cytology for diagnosing spinal lesions were 94.73%, 96.15%, 97.29%, 92.59%, and 95.31%, respectively.

4. Discussion

The importance of squash cytology has increased with the availability of computed tomography and magnetic resonance-guided stereotactic biopsies [4,5]. It guides the surgeon regarding the extent of surgery and helps optimize the treatment plan until the final histopathological examination result is available. In a study conducted by Anita et al., 20 cases of spinal tumors were studied [3]. Squash cytology smears were stained with hematoxylin and eosin and toluidine blue after fixation with 95% ethanol [3]. In our study, we employed hematoxylin and eosin and Papanicolaou stain for staining squash cytology smears in all 64 cases evaluated. The diagnostic accuracy for intracranial lesions is generally higher than that for spinal lesions. However, the results of our study are comparable. Squash cytology provides valuable morphological details, such as cytoplasmic and nuclear features, as well as cytoarchitecture (e.g., rosettes, whorls, and calcification) [6-8]. Nevertheless, crushing, overstretching, and cellular overlapping may produce poor-quality smears, which limit the accuracy of squash cytology [3]. Additionally, the presence of fibrous elements and bony spicules may sometimes lead to difficulty in smearing and pose problems in accurate interpretation [9,10]. In our study, spinal lesions were distributed across all age groups, with a peak incidence in middle age, which is consistent with the findings of Bhardwaj et al. [11].

The most common intradural extramedullary (IDEM) tumor identified in our study was schwannoma, with 100% concordance. This finding is also consistent with the results of Mousumi et al., who found schwannoma to be the most common tumor in their study [12]. Several studies conducted by Jha et al. and Mitra et al. demonstrated high diagnostic accuracy of squash cytology for the diagnosis of intracranial and spinal lesions, with an accuracy rate of approximately 90% or higher [13-14]. In our study, the diagnostic accuracy was 95.31%, with four (6.25%) discordant cases. One case of hemangioblastoma was misinterpreted as astrocytoma with piloid features. The reason for this discrepancy could be a sampling error. It is possible that the squash cytology sample was taken from the periphery of the main pathologic lesion. A case of pilocytic astrocytoma was underdiagnosed as an inflammatory lesion. The squash sample was likely inadequate, containing only inflammatory cells. A case of chronic nonspecific inflammatory lesion was over-diagnosed as a small round cell tumor. A case of osteoblastoma was misinterpreted as a benign spindle cell lesion due to a sampling error. It is likely that the squash sample was obtained from the loose fibrovascular

Table 1. Breakdown of spinal lesions on squash cytology.

	Squash cytology diagnosis	Number of cases
Neoplastic (39 cases)	Meningioma	6
	Spinal ependymoma	2
	Lipoma	3
	Metastatic carcinoma	2
	SRCT	3
	Plasmacytoma	3
	Astrocytoma with piloid features	1
	Schwanomma	10
	Neurofibroma	2
	Malignant peripheral nerve sheath tumor	1
	Osteochondroma	1
	Benign spindle cell lesion	1
	Chondroblastoma with aneurysmal bone cyst	1
	Benign cartilage forming tumor	1
	Chondrosarcoma	1
	Chordoma	1
Non–neoplastic (25 cases)	Tuberculosis	17
	Chronic non-specific inflammatory lesion	5
	Epidermoid cyst	1
	Dermoid cyst	1
	Hydatid cyst	1
Total		64

stroma of the tumor. All four of these discordant cases did not undergo subsequent investigations. Under-diagnosis or over-diagnosis in squash cytology can significantly impact the patient's immediate post-operative management. The accuracy of squash cytology depends on the adequacy of the sample, which must also contain a representative area of the lesion.

In a study by Kar et al., the sensitivity, specificity, positive predictive value, and negative predictive values were 95.75%, 80.00%, 95.75%, and 80.08%, respectively [12]. These values are comparable to our study, in which specificity is higher [7]. In a study by Kishor et al., both brain and spinal lesions were evaluated together [15]. The sensitivity, specificity, and diagnostic accuracy were found to be 94.79%, 95.67%, and 95.25%, respectively, which are almost identical to the findings in our study of spinal lesions [15]. There is limited data available regarding studies conducted on spinal lesions alone. As CT/MRI were not available in all 64 cases, the sensitivity, specificity, and diagnostic accuracy of these imaging modalities could not be calculated using histopathology as the gold standard. Additionally, the availability of immunohistochemistry (IHC) in all cases would have enhanced the diagnostic reliability of the cases, as IHC is a more confirmatory technique. Increasing the sample size and incorporating more extensive IHC markers would have been beneficial in addressing these limitations of the study.

Table 2. Correlation of squash cytology with histopathological diagnoses.

Histopathology diagnosis		Number of cases	Squash cytology diagnosis	
			Number of concordant cases	Number of discordant cases
Neoplastic (39 cases)	Meningioma	6	6	0
	Spinal ependymoma	2	0	1
	Lipoma	3	2	0
	Metastatic carcinoma	2	3	0
	SRCT	3	2	0
	Plasmacytoma	3	2	0
	Astrocytoma with piloid features	1	3	0
	Schwanomma	10	0	1
	Neurofibroma	2	10	0
	Malignant peripheral nerve sheath tumor	1	2	0
	Osteochondroma	1	1	0
	Benign spindle cell lesion	1	1	0
	Chondroblastoma with aneurysmal bone cyst	1	0	1
	Benign cartilage forming tumor	1	1	0
	Chondrosarcoma	1	1	0
	Chordoma	1	1	0
	Tuberculosis	17	1	0
Non–neoplastic (25 cases)	Chronic non-specific inflammatory lesion	5	17	0
	Epidermoid cyst	1	4	1
	Dermoid cyst	1	1	0
	Hydatid cyst	1	1	0
	Hydatid Cyst	1	1	0
Total		64	60	4

Table 3. Diagnostic accuracy of squash cytology based on true positive (TP), true negative (TN), false positive (FP), and false negative (FN) cases.

Diagnosis on squash cytology	Histopathology diagnosis		Total
	Positive	Negative	
Positive	36	1	37
Negative	2	25	27
Total	38	26	64

5. Conclusion

Squash cytology is a rapid, cost-effective, and highly accurate intraoperative diagnostic method. While its role in spinal lesions remains understudied, our research demonstrates its high diagnostic accuracy for this purpose. This technique allows surgeons to effectively determine the extent of surgery and optimize immediate postoperative management. Furthermore, diagnostic accuracy can be further enhanced by considering clinicoradiological correlation when interpreting squash cytology findings. Our study underlines the valuable role of squash cytology in diagnosing spinal lesions, while also highlighting the potential for further research exploring the role of immunohistochemical markers in this context.

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Conflict of Interest Statement

The authors declare no conflict of interest.

Author Contributions: Design conception, data collection, data analysis, and preparation of draft, Diya Bajaj (D.B.); Design conception, data collection, data analysis, and preparation of draft, A.G.; Data collection and data analysis, N.Y.; Design conception, A.K.; Data collection, V.P.; Data collection, S.R.; Design conception and data analysis, J.B.; Data collection, M.S.; Data collection, K.H.; Data collection, Mu.S.; Data collection, J.P.; Design conception, M.N.S. All authors have read and agreed to the published version of the manuscript.

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